The University of Chicago

THE CONNECTION OF MILKSICKNESS WITH THE POISONOUS QUALITIES OF WHITE SNAKEROOT (EUPATORIUM URTICAEFOLIUM)

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF HYGIENE AND BACTERIOLOGY)

BY

WALTER GEORGE SACKETT

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THE CONNECTION OF MILKSICKNESS WITH THE POISONOUS QUALITIES OF WHITE SNAKE-ROOT (EUPATORIUM URTICAEFOLIUM)

WALTER G. SACKETT
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INTRODUCTION

Since the pioneer days of 1840, when hundreds of acres of our most fertile river-bottom lands and wooded slopes were still uncleared, some animal husbandmen have associated the common white snake-root, Eupatorium urticaefolium, with a disease of cattle, sheep and horses, variously designated as "milksickness," "milk sick," "staggers," "trembles," "alkali" and "slows." Later opinion has tended to confirm this view, but much of it has been little more than the expression of the personal impression of untrained observers.

As early as 1839, we have the record of Rowe[1] who fed the plant to cattle with fatal results.

Barbee,[2] in 1840, after searching for possible causes of the disease, concluded: "Nor have any circumstances in the history of 'Milk-Sick' in these districts (Ohio) enabled me to approach nearer to a discovery of its cause than that it probably has a vegetable origin. By some it would be deemed altogether unnecessary to offer anything in the support of the supposition of the vegetable origin of the disorder. This is the most popular theory and has more facts to sustain it than that which contends for a mineral poison." He had noticed that cows which were enclosed in woodland pastures developed trembles and died. His observations led him to believe that the two plants which were probably responsible were white snake-root and poison oak (*Rhus toxicodendron*). He fed a decoction of the first to a dog which showed characteristic symptoms and died in 3 hours; to another he gave a decoction of the latter plant with results similar to the first, except that the animal died in 2 hours. Barbee states that an acquaintance of his, Dr. Owen, had fed a decoction of E. ageratoides (urticaefolium) to a calf which developed trembles and died in a few hours. A fact of additional interest is that Dr. Owen had received specimens of plants identified as E. ageratoides, from farmers in Indiana who claimed that this species was responsible for trembles. Some argued that it could not be the cause of the trouble for, were that the case, all cattle which ate it should die, and this was not true. In defense of his position, Barbee advanced the idea of individual resistance among the animals, asserting that "the vital powers may resist the poisonous agency in any quantity."

Received for publication Oct. 1, 1918.

[1] Ohio State Jour., Sept. 6, 1839.
[2] Western Jour. Med. and Surg., 1840, I, p. 178.

In June, 1855, Vermilya[3] of Ashland Co., Ohio, confined 200 sheep in a pasture where white snake-root was growing along with other weeds and grass. He noticed that the animals did not eat it as long as there was any grass available. In the course of time, several sheep developed trembles, and after 6 or 8 had died and 5 others were affected, he took them away and all recovered. An old sheep was given the run of this same pasture, but refused to eat the snake-root even when tempted to do so by salt sprinkled over the leaves; it licked off the salt, but would not taste the plant. In October of the same year, an old mare was turned into this enclosure but ate the weed only when it was mixed with oats; on the 3rd day she ate some clear and died on the 4th day. One year later, Vermilya fed small quantities of it to a 7-year old mare daily until she refused it; trembles appeared and the horse died 11 days after the feeding was begun. Regarding the attitude of livestock toward the plant, this observer says, "I think that nothing will eat it when they can get plenty of other food."

It is worthy of note, in passing, that in these experiments, E. ageratoides was poisonous both in June and October, a point of interest, since some plants are said to be much more dangerous at one period of their growth than at another.

In summing up his views on milksickness, Vermilya in 1858 says: "The malady prevails to a considerable extent in many parts of our country, more particularly in new and uncultivated lands. Horses, sheep and cattle are affected by it, as well as people; indeed, people are not afflicted by it except by the use of milk taken from a cow which is diseased. It sometimes appears in old settlements, but if so, it is owing to a season not furnishing sufficient food for cattle, by which they are driven to eat articles that, in other seasons, when food was plenty, they would reject. Thus, the past season, when food was scarce, this disease prevailed considerably in various parts of the state. We have a cause that will produce the effects indicated by this disease. A species of Eupatorium, commonly called white snake-root, will produce all these effects. In materia medica, it is used for that purpose. It grows in shady, moist land, and if food is plenty, cattle will not eat it. When pinched by hunger, they will."

One of the most interesting cases on record is that reported by Jerry[4] of Madison Co., Ill. In June, 1860, he collected what he supposed to be common nettle for greens. His wife cooked them and he was the first one to taste them. He noticed at once a peculiar flavor and odor, quite different from that of nettle greens and immediately suspected that he had gathered the wrong plant. None of the other members of his family ate any, and they all remained well. The next day, however, Jerry was taken violently ill with symptoms which were identical with those of milksickness. Recurring attacks were experienced, and he did not recover completely for five years. In this respect, the history of the case corresponds very closely with those said to be acquired through the use of milk.

Later, Jerry fed a decoction of the unknown plant, which had sickened him, to his dog, and the latter became violently ill as a result. Some seven years later the "greens" were identified by Enon Sanders of Edwardsville, Ill., as E. ageratoides.

[3] Ohio State Board of Agr., 13th Ann. Rept., 1858, p. 670.
[4] Med. and Surg. Report, 1867, p. 270.

Observations of this sort by the early settlers could be duplicated almost ad infinitum, but the few examples cited serve to give an idea of the importance which was attached to poisonous plants, and more especially to white snake-root, as the cause of trembles.

The history and progress of the disease seem to have followed much the same course in both man and the lower animals, a fact which might point to a common origin or to an inter-relation of causal agents.

A most excellent historical sketch and bibliography of milksickness have been given by Jordan and Harris,[5] and there is no need for unnecessary repetition at this time, except for a few of the more salient points which are of more or less general interest.

HISTORICAL

The fact appears to be well established that the disease existed in North Carolina as early as 1778, and that by 1825, as civilization moved westward, it was well known to the settlers of Tennessee, Kentucky, Ohio, Indiana, Illinois and Michigan.

Since 1840, there has been a gradual but marked falling off in the number of cases until at the present day, it is a relatively rare occasion for a physician to be called to wait on a patient with milksickness. Whereas, in the early days there was hardly a medical man who had not had first-hand experience; today, it is the exceptional practitioner who has ever seen a case. Occasionally, however, even nowadays, outbreaks occur such as those reported by Jordan and Harris[6, 5, 7] in New Mexico, in 1907, in Altamont, Illinois, in 1908; by Walsh[8] at Morris, Ill., in 1908 (7 cases); by Clay[9] in Vermilion, Clay and Wayne Counties, Ill., in 1913 (17 cases), and by Schwarze[10] near Atwood, Ill., in 1917.

As one begins to analyze the data at hand in an attempt to account for the almost complete disappearance of trembles and milksickness, two facts stand out very prominently:

In the first place, in the pioneer days when the disease was most prevalent this entire region was heavy timberland, and in all probability cattle roamed unrestrained. The native vegetation, including poisonous species, presumably

[5] Jour. Infect. Dis., 1909, 6, p. 401.
[6] Jour. Am. Med. Assn., 1908, 50, p. 1665.
[7] Science, 1909, N. S. 29, p. 1010.
[8] Ill. Med. Jour., 1909, 15, p. 422.
[9] Ill. Med. Jour., 1914, 26, p. 103.
[10] Jour. Am. Vet. Med. Assn., 1918, 53, p. 236.

was very dense and every possible opportunity was offered to the stock to feed on these plants. Under such circumstances, trembles developed at an unprecedented rate.

In the second place, as the land has been cleared, drained and brought under cultivation, which operation is still going on, the natural range for cattle has become less and less each year, and will continue to do so each succeeding year. With the gradual elimination of the native pasture, the opportunity for ingesting poisonous plants has been reduced, with the result that trembles and milksickness have disappeared at a rate commensurate with the amount of clearing.

Another fact which probably plays a part in the decrease in the number of cases of trembles and milksickness is better diagnosis; we have every reason to believe that formerly many ailments were pronounced milksickness which today would be given an entirely different classification.

It will probably be found that more than one species of plant is capable of exciting the symptom complex termed "trembles." Further study may show different group complexes, each separate and distinct from the others and characteristic of a particular disease specific for a single species of poisonous plant.

ETIOLOGIC FACTORS

The number of poisonous plants thought to affect milk in one way or another, either by increasing or decreasing the flow, or by imparting some disagreeable flavor, objectionable color or deleterious quality is relatively large. According to Long[11] the list for the United Kingdom includes 31 different genera. Of these, Colchicum autumnale, when fed to goats, has made the milk poisonous for infants; Narthecium sassifragum is alleged to have poisoned a cow so that its milk killed a cat; Euphorbia lathyrus when eaten by goats is said to impart its poisonous qualities to the milk.

In addition to Eupatorium urticaefolium and Rhus toxicodendron, already mentioned in connection with trembles, several others have been named as standing in a possible causal relation:[5] "Lobelia inflata (Indian tobacco), Bignonia capriolata (cross vine), Apocynum cannabium (Indian hemp or Indian barley), Caltha palustris (marsh marigold), Euphorbia esula (spurge), Aethusa cynopium (fool's parsley), Psedera quinquefolia (Virginia creeper), Symphoricarpus orbiculatus (Indian current), Cicuta maculata (cowbane), and Bigelovia rusbyi" (now probably Chrysothamnus bigelovii), rayless goldenrod or rabbit brush of Colorado and New Mexico.

Poisonous mushrooms were suggested by Winans,[12] in 1840, as a possible cause of the disease. Johnson[13] in 1866 held the same view, although admitting that he had little experimental proof to fortify his position. Twenty years previously, he had mixed the expressed juice of a mushroom with milk and sugar, and when this was fed to flies, chickens and a cat, it resulted fatally. In his own words he says: "I am of the opinion that this poisonous agent is the mushroom, and whilst I am not able to demonstrate the fact from an actual test, still it is the only conclusion justified by the fact at which I have been able to arrive."

[11] Plants Poisonous to Livestock, 1917, p. 99.
[12] Western Jour. Med. and Surg., 1840, p. 191.
[13] Atlanta Med. and Surg. Jour., 1866-67, 7, p. 289.

Slack,[14] in 1854, maintained that trembles was due to a parasitic fungus like ergot which infected the vigorous swamp grasses of the lowlands, and that cattle which were pastured in such localities ate the poisonous principle along with the grass seed. To the active constituent he gave the name, "Ergdeleteria." In defense of his position he says, "the cause has been removed by clearing and cultivating the land, and draining the swamps. Wherever drainage and cultivation are vigorously pushed, the disease is no longer found; because the strong pasture grass having air and sunlight, the nidus of the fungus seeds is not furnished and the fungus, which is a poison parasite, will not grow and ergotise the seed of the grass."

It is a matter of experience that just such treatment of the land as is described above has also reduced the amount of white snake-root very materially.

Nagle[15] was also an exponent of the ergot theory (1859). His conclusions were based, in part, on limited feeding experiments in which grass from swamp was cut and fed to a cow. In the course of time trembles was produced and death followed. The meat from these animals, when fed to dogs, killed them. Nagle's description of the grass, leaves little room to doubt that there was a parasitic fungus present; in fact, it was confirmed by microscopic examination. The question hinges on whether his cows developed typical symptoms of trembles, or of ergot poisoning.

While the poisonous plant theory appears to have been the one most generally accepted as the cause of the disease, numerous other views have been advanced to explain its origin.

The mystic miasma emanating from swamps, which for years veiled the secret of malaria; the web of an innocent insect; the dew on the grass; a yeast; impure air; diseased soil; and even "remaining in the timber over night," all have received their share of consideration. A mineral, "rising out of the ground and collecting on the leaves of plants in yellow droplets" was found by the writer regarded as the cause in Tennessee as late as July, 1918. This deposit was claimed to be especially noticeable "of a morning" when the dew was heavy and if the plants were growing in the proximity of a spring. The possible relation of impure water has not been lost sight of; mineral salts which had accumulated in excess in stagnant pools from which the cattle drank have been suggested; animals are said to have licked the soil and obtained poisonous minerals in that way; minerals of a metallic kind such as those of arsenic, copper, mercury, cobalt, barium and aluminum believed to exist in the native vegetation in toxic amounts, have been exploited by their respective exponents.

During the past year, Curtis and Wolf,[16] Marsh and Clawson,[17] and Moseley[18] have published the results of experiments which point

[14] Western Lancet, Cinn., 1854, 15, p. 140.
[15] Nashville Jour. Med. and Surg., 1859, 17, p. 289.
[16] Jour. Agr. Research, 1917, 9, p. 397.
[17] Jour. Agr. Research, 1917, 11, p. 699.
[18] Med. Rec., 1917, 92, p. 428.

quite conclusively to Eupatorium urticaefolium as the true cause of trembles.

The more recent conceptions are of sufficient importance to warrant our giving them greater consideration than some of the earlier experiments, and therefore they will be discussed more in detail in the following pages.

RECENT EXPERIMENTAL WORK

Moseley,[19] in 1905, was the first to undertake anything in the way of a systematically organized study of trembles, according to our modern idea of what such experiments should be. He prepared both a milk infusion and a water decoction from fresh snake-root leaves and stems, and fed this to cats and rabbits. Both animals showed symptoms of the disease, but only the cats died as a result. Rabbits fed with the green plant developed trembles and died after 3 days. The viscera and meat from these rabbits were given to cats, trembles appearing in all, but only 1 died. A dog was given the aqueous extract and the chopped leaves and stems of plants along with milk and other food; in less than 24 hours, the dog was seized with violent trembling but recovered. A sheep was feed the green plant, of which it consumed approximately 29 ounces; it remained normal until the 3rd day when it manifested characteristic trembles and prostration; it died the next day; the necropsy showed both kidneys to be greatly enlarged. An aqueous decoction was injected subcutaneously into a rabbit, as a result of which trembles followed, but not death; subsequently, the animal was fed fresh snake-root which killed it in 5 days. In commenting on the behavior of the poison, Moseley states that his experiments with both rabbits and cats indicate that these animals may acquire some degree of tolerance for the poison.

Crawford,[20] in 1906, prepared an aqueous extract from both dried plants and those preserved in water to which sufficient chloroform was added to prevent fermentation. The extract was either fed or injected subcutaneously into rabbits, cats and dogs; 4 rabbits died as a result. He also injected the water extract of the ash from a dried plant into a rabbit with doubtful results. A lamb weighing 25 kg. was fed 58 gm. of fresh material with no other effect than a slight diarrhea. Crawford, himself, took the extract from approximately 300 gm. of the fresh plant without experiencing any of the symptoms of milksickness.

As a result of his experiments, he concludes that "it certainly cannot be said that it has been proved that milksickness is due to any constituent of Eupatorium ageratoides." Notwithstanding the fact that 4 of his 13 experiments gave positive results, he is disposed to dismiss Moseley's work with the challenge, "that it cannot be said that Moseley has even proven Eupatorium ageratoides to be a poisonous plant, much less the cause of trembles."

Moseley,[19a] in 1909, claims to have produced the same results in rabbits by feeding them aluminium phosphate as by giving them white snake-root. He has found considerable quantities of this salt both in the leaves of Eupatorium urticaefolium and in the stems of Isocoma heterophylla (rayless goldenrod). Aluminium was found in the milk of a cow fed with white

[19] Ohio Naturalist, 1906, 6, p. 463.

[19a] Moseley, E. L.: Med. Rec., 1910, 77, p. 620.

[20] Bulletin No. 121, Part I, Bu. Plant Industry, U. S. Dept. Agr., 1908.

snake-root and this milk produced trembles in cats and rabbits. Aluminium was found in the urine, liver, kidneys and muscle of rabbits fed snake-root and the meat, whether raw or cooked, produced trembles in cats. Therefore, Moseley concludes that trembles and milksickness are due to aluminium phosphate and that animals get this substance from white snake-root in the Central States and from the rayless goldenrod in New Mexico.

Later[20] he examined the butter from a farm where milksickness had occurred in the family and found it to contain 0.02% of aluminium phosphate. When this butter was fed to cats, it produced trembles in a few days. Further feeding experiments were carried out on cats with aluminium phosphate, and it was found to be most effective when given with fat meat, and less so if mixed with lean meat; administered in milk it was less active than with fat meat, while with vegetables it had no perceptble action. The use of sodium bicarbonate as a remedial measure as recommended by Walsh[8] was attended with beneficial results, and hence Moseley believed that "a part of the injury to the system produced by the aluminum was due to its producing a tendency to acidity."

Jordan and Harris[5, 6, 7] during an outbreak of so-called milksickness in New Mexico, isolated an aerobic, spore-forming bacillus in pure culture from cases occurring in both man and the lower animals. At the time, they thought that this micro-organism might be the responsible agent, and accordingly named it "Bacillus lactimorbi." Subsequent experiments with laboratory animals, in which pure cultures of the organism were both fed and injected, have produced little or no effect, and they concluded finally: "Taken as a whole the facts do not surely indicate that a special micro-organism is the cause of milksickness or trembles."

Clay[9] of Hoopeston, Ill., after losing a large number of cows and horses from trembles prior to 1914, procured two young cattle and one sheep that were healthy and that had not been in wild pasture. They were confined in a barn-lot and required to eat Eupatorium urticaefolium cut fresh at each feeding. All were dead within 3 days, the symptoms being identical with those of animals which had died previously on the natural range. He believes that the disease is due to an intoxication rather than to an infection, since in the 17 cases in man which he attended in 1913, he was unable to find Bacillus lactimorbi of Jordan and Harris either by cultural methods or by direct microscopic examination of the blood or urine.

Marsh and Clawson[17] fed sheep and cattle with both dried and fresh Eupatorium urticaefolium at Washington, D. C., and Beecher, Ill., in 1914. Their results show conclusively that the plant is toxic, and that its ingestion is accompanied by a definite symptom complex resembling that described for trembles. They believe that the poison has a cumulative effect, and that the plant loses a large part of its toxicity in drying.

Curtis and Wolf,[16] in 1916, fed fresh Eupatorium urticaefolium to 15 head of sheep; all of these developed trembles; 14 died and 1 recovered. Healthy sheep confined with the affected ones failed to show the disease; this fact is very properly considered as indirect evidence against the infectious nature of the disease. No harmful effects resulted from feeding aluminum phosphate with grain and hay; salt and soda administered with the food were without remedial value.

Within the past year (1917) Moseley[18] has prepared an ether extract from the leaves of white snake-root. The plants were gathered in a woods which had served as pasture for a cow whose milk and butter are said to have

caused milksickness in 3 members of a family, 2 of whom had died. He states that nearly all of this extract is a resin, and that when taken with food by rabbits, it affects them similarly to snake-root. The same symptoms were produced in a guinea-pig. Cats show some of the symptoms of trembles, but as a rule they will not eat enough to kill them.

The inconclusive character of some of the experiments seemed to warrant a further examination of the subject.

THE GENUS EUPATORIUM

Name of Genus.—This genus is said to be christened[21] Eupatorium after the ancient king of Pontus, Eupator Mithradates VI.

BOTANICAL CONSIDERATION

Species Description.—Eupatorium urticaefolium, Reichard (Eupatorium ageratoides, L. f.—white snake-root[22]).

Heads 8-30 flowered; involucral bracts nearly equal, in one row or but a very few of the outermost shorter. Plants smooth, branching, 0.5-1 mm. high; leaves broadly ovate, opposite, long petioled, triple nerved, not resinous, dotted pointed, coarsely and sharply toothed, thin, 7-12 cm. long; corymbs compound, flowers pure white. Rich woods, not rare.

This species thrives best in a rich, moist soil, rather light in texture, such as is found in our shaded coves or ravines and moist woodlands. Protected by the trees, the plant seems to find optimum conditions for its growth in the heavily shaded, moist areas in the vicinity of a stream. We rarely find it growing in the open, unless it is where the land has been recently cleared of timber and where an abundance of moisture is still present. Bright sunshine causes rapid wilting, and accordingly, it disappears early from unprotected areas.

Eupatorium urticaefolium is reported as occurring in the states of the United States east of the Rocky Mountains, except in Kansas, Oklahoma and Texas. West of the Rockies, its presence has not been observed up to the present time.

Number of Species.—The number of species of Eupatorium which have been observed and described in the different parts of the world, is rather astonishing, considering the fact that most of them are only common weeds, and very unpretentious at that. In the neighborhood of 400 different kinds have been noted, all but about a dozen of which are natives of the Western Hemisphere, and most of these are to be found in the warm temperate or tropical regions. Some 50 have been reported as used in one way or another in the arts and industries.

DISCUSSION OF THE ACTIVE CONSTITUENTS OF EUPATORIUM SPECIES OTHER THAN E. URTICAEFOLIUM

According to Hager,[23] "E. perfoliatum L. is native to American Southern states. The plant is used as a source of bitters. It is said to contain a glucosid, which is also called Eupatorine, a nonglucosidal principle, 0.1% of

[21] Sprengel, Kurt: Versuch einer pragmatischen Geschichte der Arzneikunde, 1800, Pt. 1, p. 634.

[22] Gray's New Manual of Botany, 7th Edit., Rev. 1908.

[23] Handbuch der Pharmaceutischen Praxis, 1900, 1, p. 1069.

volatile oil, gallic acid, tannic acid, etc., and the flowering plants an alkaloid. The leaves are used, as well as the extract.

"Also E. purpureum L., trumpet weed, purple boneset or gravel root contains a little volatile oil and the glucosid Euparine, $C_{12}H_{11}O_3$."

Several members of this genus have been used for the volatile oil which they contain; two have been employed as a source of indigo; two as substitutes for hops and one in place of tobacco. The leaves and blossoms of Eupatorium perfoliatum and E. purpureum contain bitter principles to which their reputed medicinal value has been ascribed.

Eupatorium perfoliatum, L.—The American Indians appear to have been the first to utilize Eupatorium perfoliatum or common boneset as a medicinal plant.

Anderson,[24] in 1813, reported its use among the native tribes as a successful remedy in the treatment of "intermitting and remitting" fever.

Stevens[25] and Hosack[26] reported that this species was known to be popularly used for the cure of malaria in 1803 and it was advocated also in the treatment of yellow fever.

Wilkins[27] reports successful use of the leaves in the treatment of tapeworm.

Peterson[28] believed that this plant contained some principle peculiar to itself and different from quinin, as had been previously suggested, to which its tonic and emetic properties were due. Although Peterson was unsuccessful in isolating the active constituent in a pure state by treating the leaves with water and subsequently extracting the aqueous residue with alcohol, he proposed the same "Eupatorin" for the bitter residue.

In 1854, Bickley[29] obtained a yellow, slightly crystalline substance, extremely bitter and nauseating from a cold aqueous infusion of the leaves which was subsequently concentrated, dried and treated with boiling 95% alcohol. He did not proceed further to determine the nature of the yellow substance.

Collier and Parsons[30] made an analysis of E. perfoliatum in 1879 and found a bitter principle which is described as a brown uncrystallizable substance soluble in water and alcohol, insoluble in ether.

Latin,[31] in 1880, obtained a bitter principle which yielded reducing sugars on hydrolysis. He stated that this was probably of glucosidal nature and that it was soluble in alcohol, ether, chloroform, boiling water and concentrated acids.

Franz[32] found a bitter principle in the leaves which was nauseous. Hydrolysis experiments led him to believe that what he had obtained was a glucoside.

In 1892, Shamel[33] separated what he believed to be the active ingredient in pure form. The bitter principle occurred either as a yellow resinous mass or as a yellow powder, which, under the microscope, showed globular masses of needle-shaped crystals. These were soluble in dilute nitric acid, and when the nitrate was allowed to crystallize "beautiful prisms and six-sided plates" were obtained. "An aqueous solution of these crystals injected into mice killed them in a few hours."

[24] A Dissertation on the Eupatorium perfoliatum. May 4, 1813,
[25] Med. Repository, VII, 12.
[26] Med. Essays, III, p. 434.
[27] Am. Jour. Phar., 1874, 46, p. 295.
[28] Am. Jour. Phar., 1851, 23, p. 206.
[29] Am. Jour. Phar., 1854, 26, p. 495.
[30] Am. Jour. Phar., 1879, 51, p. 342.
[31] Am. Jour. Phar., 1880, 52, p. 392.
[32] Am. Jour. Phar., 1891, 60, p. 77.
[33] Am. Chem. Jour., 1892, 14, p. 224.

The roots of this plant were examined the same year by Kaercher[34] who secured an amorphous bitter substance soluble in chloroform but which gave no reaction with either alkaloidal or glucosidal reagents.

In attempting to repeat the work of Shamel, Walter,[35] in 1900, discovered that the crystals, which the former had obtained by treating his yellow residue with nitric acid, and which he believed was the nitrate form of the active ingredient, were nothing more than crystals of pure oxalic acid such as might be expected to result from treating tannic acid with nitric acid, and which in Shamel's preparation had probably originated from the tannin that was present as an impurity. In the light of this disclosure, it would appear that the mice which Shamel injected succumbed to oxalic acid, rather than to some other form of poison present in the active principle.

The active ingredient as obtained in a purified form by Walter was a light reddish-brown residue, extremely bitter, having the formula $C_{35}H_{58}NO_{10}$. This was neither fed nor injected into animals, so we have no data on its poisonous properties.

Reasoning by analogy from other bitter principles which are known to be active ingredients of certain drugs, we are naturally led to the concluson that, here too, the active principle resides in the bitter substance, but the fallacy of such reasoning without animal experimentation is too obvious to need further comment.

Eupatorium purpureum.—Siggins,[36] in 1888, obtained an ether soluble substance from the leaves of this species which he believed to be a glucosid with a bitter taste.

In the same year Trimble, Ray and Eberhardt[37] extracted a yellow, resin-like uncrystalline substance from the roots of this plant by means of ether, but it gave none of the tests for either alkaloids or glucosides. It was named Euparin, as suggested by Lloyd[38] and given the formula $C_{12}H_{11}O_3$.

Manger,[39] in 1894, confirmed the work of Trimble.

From the analyses made on E. purpureum it appears that the leaves contain a bitter principle, glucosidal in character, and that the roots yield a crystalline nonglucosidal substance, Euparin.

THE PRESENT WORK

Frequent reference is made to the fact that trembles is most prevalent among cattle which are pastured on moist, shaded, rich land. This is where white snake-root finds conditions adapted to its growth.

We read that the disease is always worse in a dry season or late in the fall when feed is short and poor. This can be easily explained, for during such times the native grasses would be insufficient for the animals' needs, and they would eat anything green, regardless of whether it was snake-root or red clover.

[34] Am. Jour. Phar., 1892, 64, p. 510.
[35] Proc. Am. Phar. Assn., 1900, 48, p. 216.
[36] Am. Jour. Phar., 1888, 60, p. 225.
[37] Am. Jour. Phar., 1890, 62, p. 73.
[38] Am. Jour. Phar., 1876, 48, p. 331
[39] Am. Jour Phar. 1894, 66, p. 121

On the other hand, very little of the disease is experienced if the season is wet. Under such conditions, the natural range would be good and sufficient for the animals' demands, and there would be no occasion for them to eat poisonous weeds.

It is asserted that stock in one pasture or locality are affected, while the animals in an adjoining or neighboring tract are not, yet to the casual observer conditions are identical. The observations of the writer lead him to believe that a more careful study of the forage possibilities in the two localities would explain this point, for just such a circumstance was called to his attention last fall. An examination of one pasture (Morris milksick) showed practically nothing but weeds, while just across the road (disease free) there were plenty of the same kinds of weeds, but, in addition, abundant blue grass. Manifestly the cattle feeding on the one side of the road would be compelled to eat snake-root, while on the other they would not. Extracts of plants from both areas have been shown to be equally poisonous, so that the failure of the animals to develop trembles on the one tract cannot be charged to a lack of poisonous properties in a part of the plants, but rather to the failure of the cattle to eat the snake-root where grass was plentiful.

Out of such assertions as the foregoing, the belief has grown up that snake-root from one locality is poisonous while that from another is not. Neither the truth nor the falsity of this claim can be established at present.

COLLECTION OF MATERIAL

In order to determine whether such expressions as these were merely opinions, or whether experimental evidence could be produced that would justify these utterances, we endeavored, with the cooperation of the state experiment stations, to obtain green plants from widely separated regions. Unfortunately, requests for this material were not sent out until the middle of October, 1917, and by that time, many localities had been visited with heavy, killing frosts, so that the range of territory represented was not as large as we had hoped for. However, specimens were received from Alabama, Iowa, Massachusetts, Minnesota, Missouri, Ohio, Pennsylvania, Tennessee and Wisconsin.

Through the courtesy of Dr. Walsh of Morris, Ill., we learned of a most interesting "milksick" area, a moist woodland pasture, bordering a stream, where Eupatorium was growing abundantly and which has a definite history. The land in question lies on either side of the main traveled road, and it was alleged that cattle which were allowed to browse on the side of the highway invariably contracted milksickness, and some animals had died, whereas if they were permitted the range of the other side, no evil consequences ensued. The owner was so confident that his observations were borne out by facts that he had caused a limited area on the bad side to be fenced with

barbed wire so that his cows could not have access to it. The vegetation on both sides was much the same, except that the tree growth on the milksick portion was perhaps somewhat denser, and the ground here being naturally more shaded, there was comparatively little grass in proportion to the luxuriant crop of weeds. On the disease-free side, the pasture was more open, possibly the trees stood farther apart, and the grass was much more abundant, that is to say, it was a very much better pasture. The Eupatorium, if anything, was more plentiful here than across the road. Having seen the two pastures, with their respective flora, it seemed almost self-evident why the one was dangerous for stock, while the other was relatively safe, for in the first case, the animals had been forced to eat weeds, snake-root being among those present, whereas, in the second, there was plenty of good wholesome grass which they quite naturally preferred, and so did not molest the Eupatorium.

The first collections were made Oct. 20, 1917, from the milksick area only, and at that time, the plants were just coming into flower. Two weeks later, when blossoming was practically over, a second lot of material was secured from both areas. Entire plants, including roots, stems, leaves and blossoms, were gathered; they were expressed to Chicago, and immediately on arrival, the roots were removed, washed free from soil and dried; the leaves and flowers were stripped from the stems and each air-dried separately, in order to prevent any possible injury from molding. While the plants were being collected, we were constantly on the lookout for anything that might be present on the foliage in the way of a parasitic fungus, since there was the possibility of some lower form of this sort having a causal relation to milksickness, but nothing of the kind was ever observed either on Illinois material or that from elsewhere.

Greenhouse Material

Some fifty roots, from Illinois stock and elsewhere, were set in a greenhouse in the late fall of 1917. This supplied green material for feeding during the winter.

Preparation of the Crude Drug

Whenever the condition of the plants made it possible and the size of the sample justified it, the roots, stems and leaves were dried separately, and as soon as they were thoroughly air-dry, were prepared for extraction by reducing them to a No. 30 U. S. P. powder by grinding in a suitable mill and finally by passing them through a 30 mesh sieve. Such powders were stored in glass stoppered bottles in subdued light.

Preparation of Extracts

During the course of this study we have employed several different methods of extraction, all having for their ultimate object, the separation of an active, poisonous principle. As solvents we have used 95% alcohol, 70% alcohol, physiologic salt solution (0.85% NaCl in distilled water), and a mixture of ether, chloroform and ammonia.

Alcoholic Extracts

Fifteen gm. of the No. 30 powder, an amount slightly in excess of the quantity of the leaf powder required to kill a rabbit as shown by feeding experiments, were moistened with 95% alcohol and packed firmly into a glass percolator of approximately 350 c c capacity. Enough 95% alcohol was then

added to saturate the mass and leave a thin layer on top. It was allowed to macerate in this condition for 48 hours. At the end of this time, 150 c c of alcohol were added and the percolation allowed to proceed at the rate of about 60 drops per minute. When this alcohol had all run through, more was added and the percolation continued until the drug was exhausted or until the percolate had only a very pale green color; usually 200 c c of alcohol were sufficient to accomplish this.

From this point, two different procedures have been followed to reduce the tincture, obtained above, to the consistence of a heavy paste: Either the alcohol was allowed to evaporate in the breeze from an electric fan, or the alcohol was distilled off under reduced pressure at a temperature under 35° C., the thick liquid transferred to an evaporating dish by means of a small quantity of 95% alcohol and the whole finally concentrated by the electric fan.

The solid extracts prepared by either of these methods are black in color, waxy or gummy in character, of a bitter taste which is very persistent, slightly pungent and possessing an aromatic or resinous flavor.

SALT SOLUTION EXTRACT

The salt solution extract was prepared in much the same way as the alcoholic, except that the period of maceration was reduced to 6 hours and the percolation carried out with physiologic salt solution until the percolate was practically colorless. This yielded a dark brown liquid of approximately 200 c c in volume, which was reduced to a thick syrup by boiling under reduced pressure at a temperature below 40° C. The heavy, dark brown liquid was transferred to an evaporating dish from the distilling flask with a minimum quantity of distilled water, and evaporated by means of an electric fan to a resinous, doughlike mass, shiny and almost black in color. The quantity of solid material which was yielded to salt solution was considerably greater than that obtained with 95% alcohol as the menstruum. The residue was rather pungent, with a tendency to pucker the tongue, rather than possessed of a bitter taste, and with a slight aromatic flavor.

ETHER-CHLOROFORM EXTRACT

For the extraction with ether, chloroform and ammonia, an infusion method was employed. This consisted in treating 15 gm. of the No. 30 powder in a 500 c c Erlenmeyer flask with a mixture, composed of 115 c c of ether and 35 c c of chloroform for 10 minutes in a shaking machine. Five c c of a 10% ammonia solution were then added to the mixture and the shaking continued for 2 hours. At the end of this time, the infusion was carefully poured off through absorbent cotton and then filtered through paper (S and S No. 597). This gave a beautiful, clear, dark green liquid which was allowed to evaporate spontaneously, yielding a very dark green residue, waxy in character, slightly bitter, somewhat aromatic, but with no decided flavor. The bulk of the residue was very much less by this method of extraction than with either of the two preceding, amounting in many cases, where leaf powder was used, to less than one-half gram. The bulk of a residue, where this is to be fed in its entirety to laboratory animals at a specified rate and in a given length of time, is an item which must be taken into consideration in preparing these crude extracts, since more or less difficulty is apt to be experienced in administering these even if given in pills or capsules.

FEEDING EXPERIMENTS

The work of Marsh and Clawson,[17] and more recently that of Curtis and Wolf[16] indicate that sheep and cattle fed on Eupatorium urticaefolium, experimentally at least, develop trembles and eventually may die. That there is some rather intimate causal relation between this weed and the disease, there can be no longer any reasonable doubt, and on this ground, there is ample reason for including this malady among those which are considered as being due to food poisoning. Under this classification, however, there are several possibilities.

In the first place, specific pathogenic micro-organisms may be present on the plants which for some unknown reason have selected this particular species as a host, and when ingested by the lower animals, they develop a toxin which produces the symptoms observed. It will be recalled that B. lacti-morbi was mentioned at one time in this connection by Jordan and Harris;[5] however, at present, they are disposed to attach less weight to their findings than formerly.

In the second place, there is the question of micro-organisms growing in and on the plants and there producing poisonous substances in the tissues either thru their own metabolism or as a result of cell destruction and disintegration in the plant. If this condition existed, we should almost certainly expect to find some external evidence manifesting itself as a plant disease. While no less than nine different parasitic fungi have been reported by Farlow and Seymour on this species of Eupatorium, the writer has taken careful cognizance of this point when collecting material, and nothing has ever been observed.

The third possibility which may be offered in explanation of this relation, and the one with which the present work is concerned, maintains that there is present in the plant itself some active poisonous principle, perhaps in the nature of an alkaloid or glucosid, which may be responsible for the trouble. That this contention is well founded, at least in so far as it pertains to rabbits, is borne out by feeding experiments conducted during the past 6 months.

Rabbits, guinea-pigs and cats have been used as the experimental animals. The first have been fed the green plants, the dried powders and the different extracts; the guinea-pigs received the last two only, and the cats were given the viscera and meat from the rabbits that died.

The fresh Eupatorium was fed by itself as long as the animals would eat it, and when they refused, it was chopped very fine and mixed with either ground carrots or celery or cabbage. The plant powders and residues were also mixed with ground vegetables. The extracts were given invariably in No. 5 gelatin capsules, and an effort was always made after administering these to get the animals to eat something at once in order to insure the greater part of the dose being swallowed.

FEEDING EXPERIMENTS WITH FRESH MATERIAL

Exper. 1.—Oct. 23, 1917, Belgian Hare 1, male, weighing 1,405 gm. was given 50 gm. of fresh leaves and stems of white snake-root which was gathered at Morris, Ill., from a milksick area on Oct. 20, 1917; some of this was eaten, but not all. Oats and water were available.

Oct. 24: Weight 1,455 gm. More fresh leaves were offered in the afternoon, and some were eaten when they were first placed in the cage; the dried material from the preceding day had not been eaten; oats and water were available.

Oct. 25: Weight 1,340 gm. The rabbit was offered fresh Eupatorium but ate nothing all day. In the evening it sat in the back of the cage, in a more or less humped up position with its head and nose extended; its breathing was short and jerky; and it kept its body swaying from side to side as if it were uncomfortable.

Oct. 26: The rabbit died during the night and the necropsy findings were as follows: The heart was normal in size, light in color, and the vessels were slightly injected. The lungs were somewhat hyperemic. The liver was hyperemic in patches, mottled with clay colored spots, with nutmeg appearance—soft, lobules very prominent, liver not enlarged. The spleen was normal except for the distal end which was dark, due probably to the position in which the animal was lying after death. The kidneys appeared normal. The stomach was full and the intestines and mesenteries were normal. The bladder was distended with urine. No odor of acetone was apparent.

The heart blood and tissues from the liver and spleen were plated in standard agar. All plates were sterile after 24 hours at 37 C.

Exper. 2.—Rabbit 2 was a Belgian hare weighing 1,270 gm. On Oct. 23, 1917, it was given 50 gm. of fresh, green Eupatorium of the same lot as Rabbit 1, but it ate none. Oats and water were given after it refused the weed.

Oct. 24: Weight 1,294 gm. It was again offered 50 gm. of fresh Eupatorium, but none was eaten. After going without food all day it was given oats and water at night.

Oct. 25: Weight 1,310 gm. None of the dry snake-root which was left in the cage from the two preceding days was eaten. No other food was given.

Oct. 26: Weight 1,340 gm. Dried material was offered, but in spite of its apparent hunger it refused to eat. From Oct. 27-30, no effort was made to get it to eat Eupatorium — oats and carrots being fed.

Oct. 29: Weight 1,330 gm. Condition was normal.

Dried snakeroot with a very small amount of oats and carrots was given on Oct. 31, Nov. 1 and 2; almost none of the weed was eaten.

Nov. 3: Weight 1,330 gm. By this time it became apparent that it was useless to endeavor to induce this rabbit to eat the plant by itself, either green or dry, and accordingly 1 gm. of the powdered leaves* from the milksick area was given to it mixed with ground carrots. None was eaten.

Nov. 4: Weight 1,295 gm. A similar mixture of carrots and leaf powder was offered, but none was eaten.

Nov. 5: Weight 1,275 gm. Having failed to get the rabbit to eat any appreciable amount of the carrot mixture, we next tried mixing 0.5 gm. of the leaf powder with chopped cabbage, but with no greater success.

Nov. 6: Weight 1,222 gm. About two-thirds of the cabbage mixture was eaten over night, but when offered a fresh lot, it was refused. Altho losing in weight continually, the rabbit appeared perfectly normal. No other food was given.

Nov. 7: Weight 1,177 gm. Cabbage mixture containing 1 gm. of leaf powder was prepared, but none of it was eaten.

Nov. 8: Inasmuch as the rabbit now refused to eat practically everything which contained the Eupatorium powder, the experiment was discontinued.

* The powdered leaves are somewhat acrid to the human taste, causing the mouth and tongue to smart and burn slightly; the flavor is not unlike that of dried alfalfa or clover leaves, and not at all disagreeable. Apparently the rabbit's sense of taste discerns something much more objectionable than the human.

The only deduction that can be drawn from this experiment is that the quantity of snake-root, both green and dried, which the rabbit consumed, was so very small that it was without deleterious effect.

Exper. 3.—Fresh material from the greenhouse, obtained from the roots previously mentioned, was used in this experiment. There were not enough plants from any one locality to permit us to feed that exclusively for any length of time; however, occasionally there was sufficient to furnish material for 2 or 3 days; again, it was often necessary to give a general mixture, so that no attempt was made in this case to draw any inference as to the respective poisonous properties of the plants from the different localities.

Approximately 10 gm. green weight was given at each feeding. Rabbit 8 seemed to be very fond of the green stuff and ate leaves and stems with great relish. The first crop lasted 15 days, during which period 153 gm. were consumed. In the beginning, the rabbit weighed 1,752 gm. and had a temperature of 102.9 F.; after 15 days, its weight was 1,758 gm. and its temperature 103.3 F. In addition to the green Eupatorium, it received a supporting ration of carrots and oats. Neither its weight nor temperature at any time varied from the normal enough to suggest anything out of the ordinary, and at the close of this first part of the experiment, the animal appeared to be in as sound condition as ever.

A second crop became available March 11, 1918, but for some unexplainable reason the rabbit refused it unless mixed with other food. About 15 gm. green weight per day were fed. The supply lasted until March 26, fifteen days, during which time 198 gm. were given, of which possibly 150 gm. were eaten.

A third lot was fed with ground carrots, beginning April 4, at the same rate as the last, and continued until April 13. Approximately 85 gm. were eaten at this time.

On April 13, the rabbit refused to eat and sat humped up most of the time; it kept its body swaying from side to side practically all of the time as if uncomfortable, and exhibited a peculiar jerking of the head; at the same time a diarrhea developed. By 7:30 p. m., its breathing had become short and jerky; it seemed to sink into a semi-coma from which it would arouse periodically, throwing its head up and backward. It was still alive the next day, but prostrate, lying on one side with its head thrown back. A partial paralysis of the head and neck had developed, placid in type, but it was still able to raise its head partly. It could not get up on its fore feet, altho the hind parts were still under control. It died at 6 p. m.

The heart showed the right auricle and ventricle dilated as well as the vessels. The lungs were normal. The liver was clay-colored and hemorrhagic. The spleen was normal, while the kidneys were clay-colored, suggesting fatty degeneration; many punctiform hemorrhages were also present. The mesenteries were hyperemic.

Kidney: Glomeruli distinctly hyperemic; capsular space normal size, contains protein granules. Epithelial cells of convoluted tubules are swollen, often filling the entire lumen. The cytoplasm contains many small granules. When stained for fat, all of the epithelial cells of the convoluted tubules are found to stain a deep orange color. The fat droplets are large and more abundant in portions of cells which border basement membrane; similar fatty changes in the loops of Henle, but are not present in collecting tubules.

Liver: Liver cords are poorly defined; cells are large and obliterate the sinusoids. The cytoplasm of liver cells is everywhere granular and many small vacuoles are seen in it. Fat stain reveals many small fat droplets in the liver cells of the peripheral one-third to one-half of liver lobules. No similar changes are noted in cells at center of liver lobules

Heart: The fibers are slightly more granular than usual and few small vacuoles are seen in them. No other definite change observed. Muscle fibers everywhere contain many small fat droplets.

Poured agar plates were made from heart blood, liver and kidney. These were sterile after 24 hours at 37 C.

The result of this experiment would seem to confirm that of Exper. 1, in so far as the ultimate poisonous action of the plant is concerned, although the total green weight consumed, which amounted to some 388 gm., was considerably in excess of that in Exper. 1. It should be borne in mind, in this connection, that the greenhouse material was new growth and very succulent, while that fed last fall was mature, ripened tissue from out-of-doors, collected late in October, at blossoming time, and in the latter case, the water content was entirely different, so that on a dry basis there was probably not so much difference as these figures would seem to indicate.

Again, there is the question of the plant being less poisonous at one period of its growth than at another, a consideration which cannot be lost sight of since it is known that with some species such as Aconitum Napellus L, or Monkshood, the plant is but slightly active when very young, and most active just before flowering, and at minimum activity when the seeds ripen.

Again, the animal may have developed a certain degree of tolerance for the drug, but eventually, the protective mechanism, whatever that may have been, gave way under the repeated administration of the plant.

FEEDING EXPERIMENTS WITH LEAF POWDER

Exper. 4.—The Eupatorium fed in this case was collected at Morris, Ill., Oct. 20, 1917, from a milksick area. The general plan of the experiment was much the same as that of Exper. 2, except that no other form of the drug was fed than the leaf powder. Two gm. of the No. 30 powder were given each day with oats, ground carrots or other vegetables. Some of the time all of the dose was eaten, while again, only a portion, so that the amount estimated to have been consumed is, at best, only an approximation.

Rabbit 2-a, weighing 1,760 gm. was given the first feeding on Nov. 7, 1917. It remained normal so far as weight and behavior are concerned until Nov. 26, when it stopped eating and its weight dropped to 1,599 gm., with a temperature of 102.9 F., a little under normal for this animal. It appeared very sick and sat in the characteristic humped-up position as if in pain. The respiration was short, fast and jerky. Toward the middle of the afternoon, it manifested further characteristic symptoms; passing into a semi-conscious condition, it would periodically arouse from this with a jerk; this was repeated 2 or 3 times per minute thruout the afternoon. Less frequently, possibly every 3 minutes, it would change its position, end for end, evidently endeavoring to seek a more comfortable posture. All of this time the head and nose were kept extended. At 9 p. m. it was still alive, but the head and thorax were lying on one side, prostrate, while the hind parts were still upright. When the animal was touched, it was still able to assume an upright position, but was unable to maintain it for any length of time, and at once fell over on its side completely prostrated. No effort was made to regain its former position. The next morning, Nov. 22, it was found dead.

The lungs were normal. The heart vessels were dilated. The liver was not enlarged, but showed marked, light, clay-colored areas; the lobules were very clearly outlined against the hyperemic tissue. The stomach, intestine and mesenteries were distinctly hyperemic. The kidneys appeared normal. The bladder was distended with urine and the stomach was full.

Kidney: The glomerular tufts are small and very markedly hyperemic; the capsular spaces are slightly enlarged and contain protein granules. The intertubular capillaries are greatly distended. All cellular structure of the convoluted tubules is lost, and the cells are so enlarged that no lumen of the tubule is visible. The cytoplasm of the cells is granular, and vacuoles are present, particularly at the periphery of the tubules.

Liver: The liver cords are not well preserved, and the sinusoids are lost. The outlines of the liver cells are indistinct particularly in the peripheral two-thirds. In this part of the lobule the cell nuclei have stained very poorly. The sinusoids contain some red blood cells. The cytoplasm of the liver cells is granular and contains many vacuoles particularly in the peripheral half.

Heart: The outlines of the cells are indistinct. Otherwise the fibers appear normal except for some irregularity in the staining of the nuclei.

Poured agar plates were made from heart blood, liver and kidney, but all were sterile in 24 hours at 37 C.

Our estimate of the amount of leaf powder actually consumed by this rabbit is about 17 gm. Death occurred on the 13th day.

Exper. 5.—Rabbit 3, weighing 1,797 gm., received daily, beginning Nov. 9, two gm. of leaf powder administered as in the preceding case. On Nov. 13, after the feeding had been in progress only 4 days, the animal became sick and failed to eat. All of the symptoms previously mentioned, such as crouching posture, short, jerky breathing, flaccid paralysis of the head and neck appeared, and the rabbit was dead by the morning of Nov. 14.

This is one of the most rapid actions that we have obtained in any of the animals that have received the leaf powder. We estimated the amount of the powder that was actually eaten at 5 gm. Death took place after 5 days.

The heart and lungs were hyperemic, the vessels of the former being dilated. The liver was hyperemic—nutmeg in appearance, soft, but not enlarged. The abdominal viscera and mesenteries were decidedly hyperemic. The spleen was dark. The kidneys were lighter than normal, clay-colored.

Kidney: Glomeruli small, cellular; capsular spaces are large and contain many protein granules indicating albuminuria. Epithelium of convoluted tubules distinctly granular; the outline of the cells is irregular and indefinite and the cells are largely desquamated.

Heart: Fibers of heart muscle contain numerous small vacuoles (fatty degeneration). Nuclei of muscle fibers present a varied appearance; some deep staining and pyknotic while others have distinctly irregular and broken outlines.

Liver: Sinusoids distended with red blood cells and liver cells throughout entire lobule contain many vacuoles. These vacuoles are numerous, each cell containing 6 or 8 visible vacuoles some of which are almost as large as the nucleus of the liver cell.

Spleen: No demonstrable changes.

Poured agar plates were made from the heart blood, liver blood, liver and kidney. All were sterile after 24 hours at 37 C.

Exper. 6.—Rabbit 4, weighing 1,966 gm., was fed 2 gm. of leaf powder daily, mixed with chopped vegetables as in the previous experiments, for a period of 8 days. On the 8th day it ate practically nothing and died on the 9th day, Nov. 18.

The symptoms manifested on the last day were in all respects the same as those already described, except that there was a slight diarrhea.

During the 9 days, the rabbit consumed approximately 10.5 gm. of the leaf powder.

The vessels of the heart were dilated. The lungs were somewhat hyperemic. The liver presented the typical nutmeg aspect and was hyperemic, mottled with lighter areas. The stomach was full. The mesenteries were hyperemic and the kidneys as well as the spleen were normal.

Kidney: The glomerular tufts are contracted and hyperemic; the capsular spaces are enlarged and contain many protein granules. The lumen of the convoluted tubules appears to be entirely closed due to the greatly enlarged and irregular epithelial cells. The outlines of the cells are scarcely visible, and the cytoplasm is granular and contains many small granules. The intertubular capillaries are distended with red blood cells.

Liver: The liver cords are poorly defined and the sinusoids are distended with red blood cells. The liver cells are somewhat larger than normal and the cytoplasm contains many large and small vacuoles. The nuclei appear to stain equally well thruout the lobule.

Heart: The muscle fibers contain numerous small vacuoles.

Spleen: The spleen appears to be normal.

Poured agar plates were made from the heart blood, liver and kidney, but all were sterile after 24 hours at 37 C.

Exper. 7.—Rabbit 5, a pregnant female, weighed 2,222 gm. She had a ravenous appetite, doubtless due to her condition, and on the 1st day, Nov. 20, she ate 3 gm. of leaf powder mixed with oats. By the next day she seemed to have developed a dislike for the material and upset the dish repeatedly which contained the oats and snake-root. Finally she ate, possibly, one-half of the dose. On the 3rd day, she refused all food, and by 5 p. m. she was very sick and hardly able to sit up. She appeared to be hungry and made several attempts to eat, but failed almost completely. By evening, she was prostrate, unable to sit up and died during the night.

The thoracic viscera were normal. The liver was uniformly hyperemic and presented the nutmeg aspect. The left kidney was somewhat enlarged, the right, normal; both were slightly hyperemic and yellow gray in color. The spleen was normal. The mesenteries and intestines were normal.

Kidney: The glomerular tufts are somewhat contracted and are extremely hyperemic; the capsular spaces are enlarged and contain but little protein material. The epithelial cells of the convoluted tubules are enlarged so that they fill the lumen of the tubules almost completely. The outlines of the cells are indistinct and the cytoplasm is granular and contains many small vacuoles. The intertubular capillaries are distended with red blood cells.

Liver: The liver cords are fairly well defined; the sinusoids contain some red blood cells. The liver cells are enlarged and the nuclei in the peripheral third stain poorly. The cytoplasm contains many vacuoles especially at the margin of the lobule.

Heart: The muscle fibers appear normal.

Spleen: The spleen appears normal.

Poured agar plates were made from the heart blood, liver, spleen and kidney, but all were sterile after 24 hours at 37 C.

A very liberal estimate of the leaf powder eaten in this case would be 4 gm. and death followed in 3 days. This is the most rapid action that was obtained in any of the leaf powder experiments.

Exper. 8.—The picture which this experiment presents is essentially the same as that given by the preceding.

Rabbit 6, weighing 1,645 gm., was fed the leaf powder with chopped vegetables, and on the 3rd day it seemed rather listless and ate but little. Here, too, as in practically all of the cases previously described, the animal seemed to be very hungry and came to its food as if possessed with a ravenous appetite, but after the first mouthful, all desire for food seemed to vanish. A slight diarrhea developed. On the next day, Nov. 13, it appeared better and ate a little. By Nov. 14, it manifested typical symptoms of Eupatorium poisoning and was dead by the morning of the next day, Nov. 15.

The heart vessels were dilated. The lungs were normal. The stomach was gorged. All of the abdominal viscera were hyperemic, particularly the mesenteries. The liver presented the characteristic nutmeg appearance and was uniformly hyperemic; not enlarged. The kidneys and spleen were normal. The bladder was distended with urine.

Kidney: The glomerular tufts are very much contracted and hyperemic. The capsular spaces are enlarged and contain many protein granules. The epithelial cells of the convoluted tubules are but slightly swollen and the lumen of the tubules is rather well defined.

Liver: The liver cords are fairly well preserved; the sinusoids are distended with red blood cells. The cytoplasm of the liver cells is very granular and contains a few vacuoles.

Heart: The muscle fibers contain many very small vacuoles, but are otherwise normal.

Poured agar plates were made from heart blood, liver and kidney, but all were sterile after 24 hours at 37 C.

This rabbit did not consume to exceed 4 gm. of the leaf powder and died 6 days from the time the feeding began.

It should be mentioned, in passing, that all of the animals were given plain oats, in quantity sufficient to maintain their normal weight, provided they ate them, but only after ample opportunity, 6-8 hours, had been offered them to eat the powder-vegetable mixture. It was clearly apparent in every case that there was something objectionable to the rabbit about the powder, for as a rule they ate it very reluctantly.

Exper. 8.—Control.—In order to make certain that our experimental animals were receiving the proper amount of food and the necessary combinations to maintain their normal weight under perfectly normal conditions, Rabbit 7, weighing 1,794 gm., was fed as a control. It received the same ration as the other rabbits, except for the fact that its food contained none of the Eupatorium. On Nov. 22, which terminated the experimental period for all of the rabbits that were fed leaf powder, this control weighed 1,775 gm., or 15 gm. less than at the beginning, a difference which falls easily within the normal daily range.

In view of the facts, that this animal which received no leaf powder, remained perfectly well and maintained its weight practically constant throughout the duration of the experiment, and whereas all 5 which ate the Eupatorium died in from 3-13 days with uniformly typical symptoms, the conclusion seems warranted that the latter came to their death as a result of Eupatorium poisoning.

FEEDING EXPERIMENTS WITH ALCOHOLIC EXTRACTS OF EUPATORIUM URTICAEFOLIUM LEAF POWDER

Exper. 10.—The solid alcoholic (95%) extract, prepared as described elsewhere, was fed to Rabbit 14 at the rate of one capsule per day for a period of 9 days. Each capsule contained an average of 161.65 mg. of the extract, which corresponds to approximately 2.8 gm. of the leaf powder, or for the whole time, the animal received extract equivalent to about 25 gm. of leaf powder.

The feeding was begun Feb. 2, 1918, on which date the rabbit weighed 2,055 gm., temperature 103.8 F. It remained normal until Feb. 13 when it stopped eating; weight 2,033 gm., temperature 102.8. It was very sick on the 15th, with a temperature a little under normal, 100.5. The body was relaxed, the head lopped to one side, placid paralysis of the head and neck. It died before the morning of the next day, Nov. 16.

The heart was enlarged, dilated and hyperemic. The lungs were somewhat hyperemic. The liver was hyperemic and cream-colored in patches. The kidneys were lighter in color than normal with many punctiform hemorrhagic spots visible through the capsule; these were suggestive of infarcts. The intestines and mesenteries were hyperemic.

Kidney: The glomerular tufts are somewhat shrunken and hyperemic. The capsular spaces are about normal in size and contain many protein granules. The cells of the convoluted tubules are greatly swollen, indistinct and irregular in outline and practically close the lumen of the tubule. The cytoplasm is very granular and contains many small vacuoles.

Liver: The liver cords are fairly well preserved at the center of the lobule but are lost in the peripheral half. The nuclei in the liver cells of the peripheral half of the lobule do not stain at all or only feebly. The sinusoids are also lost in this part of the lobule. The cytoplasm of the enlarged liver cells is granular and contains many small vacuoles.

Heart: The fibers are poorly outlined and are slightly more granular than normal. The cytoplasm contains many very fine vacuoles.

Poured agar plates were made from heart blood, liver and kidney, but all were sterile after 24 hours at 37 C.

Exper. 11.—As in the preceding experiment, the solid alcoholic extract, prepared from leaves gathered near Morris, Ill., was fed to Rabbit 15, beginning Feb. 17, 1918. At that time, the animal weighed 1,512 gm., and its temperature was 103.3 F. One No. 5 capsule was given each day for 18 days, an amount corresponding approximately to 28 gm. of the dry leaf powder. The rabbit appeared normal until the 18th day when it stopped eating. Weight 1,476 gm., temperature 102 F. By the next day, March 8, it was very weak and could be pushed over easily, but was neither prostrated nor paralyzed yet. It died on the next day with typical symptoms as previously noted, 20 days after the feeding was begun.

Punctiform hemorrhages were present in the thymus gland. Lungs were normal. The heart had a pale, light, cooked appearance. The stomach was full. The kidneys were lighter colored than normal—claylike. The liver was hyperemic with nutmeg appearance. The spleen was normal. The bladder was not entirely filled; urine neutral to litmus.

Kidney: The glomerular tufts are greatly contracted and hyperemic; the capsular spaces are not enlarged, but contain protein granules. The epithelial cells of the convoluted tubules are swollen, irregular and nearly close the lumen of the tubule; the cytoplasm is granular and contains many small vacuoles. The intertubular capillaries are distended with red blood cells.

Liver: The liver cords are well preserved and the sinusoids are distinct but distended with red blood cells. The nuclei stain uniformly thruout the lobule; the cytoplasm of the liver cells contains many vacuoles, particularly those of the peripheral third.

Heart: The muscle fibers contain many small vacuoles.

Poured agar plates from heart blood, liver and kidney were sterile after 24 hours at 37 C.

The results of the last two experiments indicate very clearly that the leaves of E. urticaefolium contain an alcohol soluble active principle which is capable of causing death when fed to rabbits.

Exper. 12.—The alcoholic extract fed in this instance was prepared from the dried stems and leaves of material received from Massachusetts; in fact, it was mostly brown, frosted stems with very little leaf tissue. Rabbit 9, weighing 1,380 gm., temperature 103.7 F. on Jan. 23, 1918, received this extract without ill effects until the equivalent of 60 gm. of the stem-leaf powder had been taken. At this point we were forced to discontinue the experiment since the rabbit had developed an infected jaw several days previously, as a result of which it died on Feb. 9, eighteen days after the experiment was begun. Up to the time of death, the animal had manifested none of the symptoms of Eupatorium poisoning, and the necropsy failed to show anything characteristic. The bacteriologic findings indicated that death was probably due to a general septicemia.

Up to the time this jaw infection occurred, stem extract, corresponding to approximately 30 gm. of the dry powder, had been given with negative results, and at the time of death the equivalent of 60 gm. had been consumed.

Taking into consideration the fact that no symptoms of poisoning were manifested at any time during life, and that the postmortem examination showed nothing characteristic, we are reasonably safe in concluding that the extract from the Massachusetts stems contained very little, if anything, that was poisonous for this rabbit in the quantity which was taken, representing more than 2 times the amount of the Morris leaf powder that was necessary to kill.

It should be remembered in connection with this material that it was largely dried stems, and subsequent tests have shown that stem extracts, even from Morris plants, are not poisonous for rabbits, at least when 3 times the lethal dose for leaf extract is administered.

Exper. 13.—Up to this point, our exeriments have dealt only with rabbits; it seemed worth while to see what effect our different preparations might have on guinea-pigs. Accordingly, Pig 1 was fed on the alcoholic extract, similar to that given to the rabbit in the preceding experiment, that is, from Massachusetts stems. The dosing began Jan. 25, 1918, and was discontinued Feb. 28, after 33 days, during which period the animal had taken extract corresponding to 110 gm. of the dry powder, an amount equivalent to almost one-fourth of its body weight. At no time was there any indication of any abnormal condition, and accordingly it appears that the alcoholic extract of Massachusetts stems is equally as harmless for guinea-pigs as for rabbits.

FEEDING EXPERIMENTS WITH PHYSIOLOGIC SALT SOLUTION EXTRACTS OF EUPATORIUM LEAVES

Exper. 14.—The large amount of soluble material which the leaves yield to extraction with physiologic salt solution complicates considerably the problem of feeding the extract to laboratory animals. In the first place, as mentioned before, it is often impossible to get a rabbit to take more than one capsule at a time. This is particularly true when the material administered contains a bitter principle, such as is present in our extracts. In the second place, when the dosage has to be strung out over a long period, for the reason just mentioned, and it is necessary when such bulk has to be given, questions of tolerance and elimination always enter in as complicating factors.

Recognizing these as possible sources of error in drawing conclusions, but having at our disposal at that time no method of concentrating the active principle from the salt solution extract, we, nevertheless, fed Rabbit 7 with the solid extract corresponding to 24 gm. of leaves. This was administred in 37 doses over a period of 36 days. The experiment was discontinued at this point, owing to the fact that the animal had developed sniffles from which it died 2 days later.

The extract seemed to have a rather drastic action on the intestine, due possibly to the sodium chlorid, for thruout the feeding there was more or less diarrhea. No symptoms of Eupatorium poisoning were observed at any time, and the necropsy findings failed to show anything at all characteristic. It is to be regretted that the rabbit died from a secondary cause after being carried for nearly a month and a half, during which time it had received daily doses of the water extract.

So far as the results of this experiment go, it appears that the salt solution extracts nothing from the leaves in the way of an active principle which is injurious for rabbits.

FEEDING EXPERIMENTS WITH THE ETHER-CHLOROFORM-AMMONIA EXTRACT OF EUPATORIUM LEAF POWDER

The most convincing and uniform results that we have obtained in all of our work have been secured with the ether-chloroform-ammonia extract of leaves from the Morris plants, gathered at blossoming time. Among the several reasons that may account for this, the following may be mentioned:

1. The poisonous principle may be more readily soluble in this solvent complex than in the other solvents employed.

2. This solvent removes less inactive material to dilute the poison than the others.

3. The bulk of the extract being so much less, it has enabled us to give the active principle in larger and more concentrated doses in a shorter time.

4. The solvent may have converted the poison into a form more readily absorbed by the animal cells, that is, into a free state.

In practically every case, it was possible to put the entire solid extract from 15 gm. of leaf powder into 3, and, at most, 4 No. 5 capsules, and unless otherwise stated, this has been the regular procedure adopted thruout the next few experiments. One capsule was given every 24 hours until the extract from 15 gm. of leaf powder had been given.

Exper. 15.—Rabbit 13, weighing 2,144 gm., temperature 102.8 F., received one capsule per day for 3 days, beginning Feb. 14, 1918. On the 3rd day, considerable difficulty was met with in getting it to take the dose. We have observed this same thing since then in a number of cases, namely, that when the animals have been sickened by the first two capsules they invariably object to taking the third. It is easily conceivable that they may have learned to associate the taste of the medicine with their condition.

This rabbit ate nothing the 4th day; it appeared weak and kept the body swaying from side to side. Its weight was a little under normal, 2,111 gm., and its temperature had fallen to 100.2. On the next day, Feb. 18, it developed a flaccid paralysis of the front legs and muscles of the neck, so that its head remained in whatever position it was placed. The hind parts were only partially paralyzed, but the animal was not able to stand or move about. Toward noon it became prostrate with its head thrown back, respiration rapid and shallow. A distinct sweetish, chloroform odor could be detected around the body. It was dead on the morning of the 19th, five days after the first dose was taken.

Sweetish, chloroform odor very pronounced. The lungs were normal. The heart was dilated and hyperemic. The liver was hyperemic with typical nutmeg appearance. The spleen was normal. The kidneys showed distinct punctiform hemorrhages. The bladder and stomach were filled. The brain and cord appeared normal.

Kidney: The glomerular tufts are somewhat contracted and hyperemic. The capsular space is enlarged and contains many protein granules. The epithelial cells of the convoluted tubules are swollen so as nearly to close the lumen. The outline of the cells is indistinct and many small vacuoles are present; the inter-tubular capillaries are prominent.

Liver: The sinusoids are distended with blood; the liver cords are not well defined. The liver cells are everywhere granular and contain many vacuoles of considerable size.

Heart: The muscle cells are poorly defined and the nuclei do not stain uniformly; the cytoplasm contains extremely fine vacuoles in great numbers.

Agar plates were prepared from heart blood, liver and kidney, but all were sterile after 24 hours at 37 C.

Exper. 16.—Rabbit 16, weighing 1,202 gm., temperature 103.7 F., was given the extract from 15 gm. leaf powder, distributed over 3 days, beginning Feb. 28, 1918. Its condition remained normal until the 4th day when its temperature dropped to 100.9 and its weight to 1,150 gm. It ate almost nothing and seemed very sick and weak; sat with its eyes half closed, ears drooped, listless and with the body humped up. It kept moving about and changing its position as if trying to find one in which it would be more comfortable. It was dead on the morning of March 4, five days after the first capsule was taken.

Choloroform odor of the body very pronounced, particularly when the skin was removed. The lungs were normal. The heart was not enlarged, but hyperemic. The blood vessels of the skin seemed to be distended more than usual. The liver was hyperemic with characteristic nutmeg appearance, though not as light in color as sometimes. The kidneys were lighter in color than normal, and one showed punctiform hemorrhages. The spleen was normal. The stomach was full and hard as seemed to be the case almost invariably. The mesenteries were hyperemic. The bladder was distended with urine which was neutral to litmus.

Kidney: The glomerular tufts are small and somewhat contracted but rather definitely hyperemic; the slightly enlarged capsular space contains protein granules; the capillaries between the convoluted tubules are more prominent than usual. The epithelial cells of the convoluted tubules are increased in size, filling the lumen nearly completely. The cytoplasm is granular; the outlines of the cells indefinite; numerous small vacuoles occur in the cytoplasm.

Liver: The cells at center of liver lobule appear practically normal; at periphery of lobules the nuclear stain is lost completely in peripheral third of some lobules; the cytoplasm of the cells is granular and their outlines are indefinite. In other lobules the changes are less marked but are always more prominent at periphery.

Heart: Only a few small vacuoles are seen in the muscle fibers.

Poured agar plates were made from heart blood, liver and kidney, but all were sterile after 24 hours at 37 C.

Exper. 16.—Thus far, all of the extracts employed in this series as well as those in the others which had proved fatal, had been prepared from leaves obtained from a "milksick" area near Morris, Ill. It will be recalled that material was also collected from a second tract adjoining the former, except for an intervening wagon road, and that altho it was reported that cattle did not sicken from eating the snake-root which grew there, the writer is of the opinion that this phenomenon was due to the fact that the stock found other forage here and did not eat the snake-root, rather than that the weed was not poisonous on this side of the road. Be that as it way, we decided to put the question to a test, and accordingly extracted 15 gm. of leaf powder from the disease-free area by the ether-chloroform-ammonia method. This was fed to Rabbit 18, weighing 1,046 gm., temperature 102.5 F., in three capsules as previously described. The animal remained normal until noon of the 4th day, March 7, when it became very weak, and by 2 p. m. it was hardly able to hold up its head. Apparently, the well recognized, flaccid paralysis was coming on, for its head was now lopped to the left side; by 2:30 p. m. it was prostrate in the cage, nose and head extended, but still able to right itself temporarily, when disturbed, after much exertion. It was dead by 3:30 p. m. There was almost no loss of weight and no subnormal temperature during the 4 days of the experiment.

The lungs were normal. The heart was hyperemic. The liver was hemorrhhagic and did not have usual nutmeg appearance, but mottled with clay colored patches. The kidneys seemed normal. The mesenteries were hypermic. The bladder was distended with urine. The brain and cord were normal. A distinct sweetish, chloroform odor was present about the body.

Kidney: The glomerular tufts are greatly contracted and hyperemic; the capsular spaces are somewhat enlarged and contain many protein granules. The epithelial cells of the convoluted tubules are somewhat swollen and irregular, but not to such an extent as to close the lumen of the tubule. The cytolasm of the cells is granular. The intertubular capillaries are distended with red blood cells.

Liver: The liver cords are well preserved and the sinusoides are clearly defined. The liver cells appear of normal size thruout the lobule and the cytoplasm contains numerous vacuoles.

Heart: The muscle fibers appear normal.

Poured agar plates from the heart blood, liver and kidney were sterile after 24 hours at 37 C.

The results of this experiment make it quite clear and unmistakable that the Eupatorium even from a "disease free" area contains an active principle poisonous for rabbits and in all probability likewise poisonous for cattle should they eat the plants in sufficient quantity.

SHAKING METHOD OF EXTRACTION VS. INFUSION METHOD

Exper. 18.—In order to ascertain whether it was actually necessary to "shake out" the poisonous principle by vigorous agitation for 2 hours, as was our practice, or whether the same result could be accomplished by simple maceration for the same length of time, 15 gm. of leaf powder, previously shown to be poisonous, were covered with the ether-chloroform-ammonia mixture and allowed to stand for 2 hours with only occasional agitation. At the end of this time the fluid was decanted and evaporated as usual. The solid extract was fed to Rabbit 11 in 5 capsules without harmful results.

In the light of later experiments, dealing with the apparently harmless action of small consecutive doses, it may be that the negative results obtained here should be attributed to the method of administration rather than to the failure of the maceration process to extract the poison. At any rate, the experiment served as a most excellent check on any possible poisonous chemical complex which might be formed by the solvents themselves. That no such complex is formed, has been demonstrated repeatedly in experiments cited later, in which the same solvents have been employed and in which the extracts obtained gave negative results.

The rabbit, used for the first part of this experiment, was rested for 15 days and then given the solidextract from 15 gm. of leaf powder by shaking the latter vigorously for 2 hours with the solvents enumerated. This was administered in 3 doses on 3 consecutive days. On the 2nd day, March 16, the animal seemed sick, and on the 3rd day, it ate almost nothing. Its weight dropped from 2,080 gm. to 1,900 gm. and its temperature from 103 to 100.2 F. By the morning of the 4th day, its head and neck exhibited flaccid paralysis, and by noon the whole body was paralyzed and the animal was unable to right itself when pushed over. It died at 2:30 p. m. on the 4th day, March 18.

Distinct sweetish, chloroform odor to the body when the skin was removed. The heart was dilated, and had a light, cooked appearance. The lungs were normal. The liver was hyperemic with usual nutmeg aspect. Both kidneys were thickly studded with punctiform hemorrhages. The mesenteries were decidedly hyperemic. The spleen was normal. The stomach was full. The bladder was distended with urine which was neutral to litmus.

Kidney: The glomerular tufts are somewhat contracted and hyperemic. The capsular spaces are slightly enlarged and contain protein granules. The epithelial cells of the convoluted tubules are swollen so that the lumen is nearly closed. The cells are irregular in outline; the cytoplasm is granular and contains numerous small vacuoles. The intertubular capillaries are distended with red blood cells

Liver: The liver cords are rather poorly preserved and the sinusoids contain many red blood cells. The liver cells are somewhat larger than normal and the nuclei stain rather irregularly throughout the lobule. The cytoplasm is granular and contains many small vacuoles.

Heart: The fibers of the heart muscle contain many very fine vacuoles.

Poured agar plates from heart blood, liver and kidney were sterile after 24 hours at 37 C.

The result of the latter part of this experiment indicates at least two things:

1. That the extract obtained by the "shaking out" method was quite poisonous for the rabbit.

2. That the rabbit did not possess a natural immunity which might explain the failure of the maceration extract to kill.

The rather quick action of the shaken extract, manifesting itself on the 2nd day, might be explained on the ground of cumulative action resulting from the material taken 15 days previously, or by assuming that the protective mechanism of the organism had been so weakened by the earlier treatment, that a given amount of the poison was able to produce more injury at this later date than would be the case in a normal rabbit. As shown by the next experiment, we have little reason for believing that anaphylaxis plays any significant rôle in the action of the poison, and therefore the explanation offered in the foregoing seems the more tenable one.

ANAPHYLAXIS

Exper. 19.—In order to determine what part, if any, anaphylaxis played in the behavior of the poison, Rabbit 20 was given one of 4 capsules prepared from the extract of 15 gm. of leaf powder on March 22, 1918. No more were given until April 5 and 6, fourteen days from the time the first was taken, on which dates 2 of the remaining 3 doses were given; the fourth, which contained about one-eighth of the total extract, was not used. The day after the first one was fed, that is, March 22, the rabbit seemed a bit sick, but recovered and appeared perfectly normal with good appetite and increased in weight until April 7, the day following the third capsule. It ate nothing and by 4:30 p. m. paralysis of the hind legs had developed while the front legs, head and neck remained active, in which respect this case differed from all previous ones. As it lay in the cage its hind legs were sprawled to either side and when it endeavored to move about, it dragged the body by the front legs, apparently unable to make any use of the hind ones. It was still alive on the 8th, but prostrate and unable to get up. It died at 10 a. m.

The heart was enlarged, dilated and hyperemic. The thymus gland was enlarged. The lungs were normal. The stomach and bladder were full. The liver was mottled with light and dark patches, hyperemic with nutmeg appearance. The kidneys were light colored with many very fine punctiform hemorrhages showing thru the capsule. The mesenteries and intestine were hyperemic. The cutaneous vessels were dilated. The brain and cord appeared normal.

Kidney: Glomerular tufts small, shrunken and hyperemic; the enlarged capsular spaces contain protein granules; cells of the convoluted tubules are swollen, granular and their outline is irregular. The intertubular capillaries are prominent.

Liver: Liver cords are fairly well preserved; the sinusoids are prominent. The cytoplasm of the liver cells is slightly more granular than usual, and small vacuoles are seen especially in cells in peripheral half of liver lobule.

Heart: Muscle fibers are fairly normal in appearance, containing few small vacuoles.

Poured agar plates made from heart blood, liver and kidney were sterile after 24 hours at 37 C.

There is nothing about the results of this experiment to indicate that there was any anaphylaxis; there was nothing in the deportment of the rabbit following the first dose after the sensitizing dose to suggest shock, and the animal developed the usual symptoms within the regular interval and died in the customary manner. It does appear, however, that this rabbit was more susceptible to the poison, either naturally or because of the effect of the first capsule, for whereas 3 capsules given in succession were ordinarily necessary to kill, here less than 2 proved fatal. It is altogether possible that the active principle exerts a cumulative effect, and that the protective mechanism of the animal was weakened by the first dose, and that in the interim of 14 days, it had not recovered sufficiently to withstand the two following doses, and therefore, reacted toward these just as it would toward any three capsules given in succession.

The rabbit weighed 1,616 gm., temperature 102.4 F. at the beginning of the experiment and 1,736 gm., temperature 101.9, two days before it died.

Dosage and Cumulative Action

Exper. 20.—As a rule, in all of our feeding experiments with the ether-chloroform-ammonia extract, the material had been administered in either 3 or 4 doses 24 hours apart. This number was selected because of the fact that the solid extract from 15 gm. of leaf powder could be accommodated in three No. 5 capsules very easily. They were given 24 hours apart because of the difficulty we experienced in getting the rabbits to take them more frequently, and with this administration, we invariably secured positive action.

The preceding experiments having demonstrated beyond all reasonable doubt that this extract contains a substance poisonous for rabbits, we next turned our attention to the effect of administering the lethal dose at different rates.

In the experiments just reported, and more especially, perhaps, in those with the fresh greenhouse Eupatorium and with the alcoholic extracts, there was some reason to suspect that cumulative action was playing a part. One might very naturally expect the same fatal result to follow the administration of the lethal dose of a poison, if it were a cumulative poison, whether given in one large dose or in several smaller ones at frequent intervals, providing, of course, that the amount of the active ingredient ingested each day was in excess of that excreted by the organism. This, however, does not necessarily follow, for it is an observed fact that a large dose of lead acetate, for example, may be taken at one time without any noticeable effect, whereas small amounts taken daily, would, in all probability, result in serious poisoning. According to Rosenau[40] "the reason for this is that where one large dose is taken only a small quantity is absorbed; the rest is swept through the intestines, but when small quantities are taken at frequent intervals practically all is absorbed and the metal accumulates in the tissue, poisoning especially the delicate nervous structures."

In order to determine the behavior of Eupatorium poison when taken in one large dose, Rabbit 19 was given the extract from 15 gm. of leaf powder at one feeding on March 20, 1918. The 2nd day it appeared rather indisposed, ate nothing and sat humped up—symptoms usually exhibited on the 3rd and 4th day by animals which have received split doses. The weight dropped from 1,660 to 1,622 gm. and the temperature from 103.2 to 102.2 F., differences which might easily fall within the individual variation. The 3rd day the rabbit seemed normal and showed no ill effects thereafter. It gained in weight steadily and is still under observation, now (5/29/18) weighing 2,185 gm. Aside from the slight indisposition exhibited by the rabbits on the day after receiving the drug, the one large dose taken at one time produced no further reaction.

Exper. 21.—By way of confirming the results obtained in the last experiment, a second animal, Rabbit 12, was given the lethal dose at one time on May 15. It appeared perfectly normal the following day, except for a slight loss in weight, and since then has never shown any indication of Eupatorium poisoning.

The results of the last two experiments seem to point to an action similar to that described in connection with lead poisoning, for manifestly there is not the same quantity of poison absorbed from one large dose as from the 3 or 4 smaller ones.

[40] Preventive Medicine and Hygiene, 3d Ed., 1917, p. 1048.

Exper. 22.—With a view to ascertaining how rapidly the active constituent was excreted and if any cumulative effect could be observed where the lethal dose was distributed over more than 3 or 4 days, we fed Rabbit 19 the extract from 15 gm. leaf powder, divided into 8 portions, over a period of as many days.

So far as we were able to detect, both by eye and with scale and thermometer, the animal remained normal throughout the duration of the experiment. Apparently, in this case, the poison in the small recurring doses was excreted as rapidly as it was ingested, and none of it was retained to produce subsequent injury to the tissue, whereas in the larger doses—3 or 4 capsules—the rate of elimination is not sufficiently rapid to prevent the absorption of injurious quantities of the active principle.

STEMS

Exper 23.—While some plants are poisonous in all their parts, the various structures differing only in the degree of toxicity, there are also those in which the active constituent appears to be concentrated in a definite part as in the seeds of Agrostemma Githago, L. (corn cockle), in the root of Sium latifolium, L. (water parsnip), and in the leaves of Conium maculatum, L. (hemlock). This is particularly true of the last mentioned in the early summer.

Cattle browsing on snake-root would be very apt to ingest more or less stem tissue along with the leaves, and therefore we prepared an extract from the stem powder of plants collected at Morris, Ill. The amount of soluble matter which 15 gm. of stems yielded to the solvent complex was less than one-third of that obtained from the leaves, and was easily accommodated in one No. 5 capsule.

The extract from 60 gm. of stem powder was fed to Rabbit 22 in 4 doses, the equivalent in extract of four 15 gm. portions of stems, on 4 different days beginning March 4. The first 3 capsules were taken at intervals of 24 hours without any perceptible change in the rabbit's condition, and 4 days from the time the third was given, the fourth was administered, but without harmful effect. The rabbit ate well and continued to gain in weight.

It appears from this that the stems contain little, if any, of the active principle compared with the leaves, since the extract corresponding to 4 times the lethal dose of leaves was fed without visible injury.

ROOTS

Exper. 24.—A bitter principle has been noted as occurring in the rhizomes and rootlets of E. purpureum and E. aromaticum. As to the presence of any active principle in the roots of E. urticaefolium, we have no knowledge.

It is easily conceivable how cattle might be poisoned in the spring by consuming whole plants, assuming the roots to be poisonous also, for at this season the ground is apt to be soft, and little effort is required to pull up the tender shoots, roots and all.

We have endeavored to ascertain the presence of an active principle in the rhizomes and rootlets of our species by feeding Rabbit 19 with the extract from 60 gm. of root powder, administered as in the preceding experiment. Here also the bulk of the extract was much less than that from the leaves. Altho the extract corresponding to 4 times the lethal dose of leaf extract was fed, there was not the slightest indication of any injurious action.

From this it appears that the underground structures are deficient in any active constituent soluble in ether-chloroform-ammonia when tested out on rabbits.

Exper. 25.—It will be recalled that in Exper. 13 a guinea-pig was fed with the alcoholic extract from Massachusetts stems and leaves, but without harmful results.

In the present case, Guinea-pig 2 was given the ether-chloroform-ammonia extract from 45 gm. of leaf powder in 9 capsules at 24-hour intervals. The pig remained perfectly well, had a good appetite and gained in weight thruout the experiment. It should be noted here that the animal received 3 times the amount necessary to kill a rabbit and that the dosing was carried out at the same rate as proved fatal for rabbits.

Exper. 26.—A leaf extract prepared from material received from Minnesota, Oct. 17, 1917, was fed in this experiment. The quantity of solid extract obtained from a given weight of this powder was practically double that from a similar weight of Morris material. The reason for this was not apparent at the time, but as will be seen from the results of the experiment, we were obviously dealing with a new set of conditions. In all, Rabbit 17 took the extract from 45 gm. of leaf powder; that from the first 15 gm. was given in 6 capsules at intervals of 24 hours, but with no effect. In the interim that followed, Exper. 22 was completed, the results of which indicated that small doses over a long period were not effective. Since the extract in the present case had been fed in 6 portions, failure to obtain positive results may have been due to a similar cause, namely, the rapid elimination of the poison. Accordingly, the experiment was repeated, and the same rabbit was given the extract from 15 gm. of Minnesota leaf powder in 3 doses on April 29, 30 and May 1, and again, that from an additional 15 gm. on May 2, 3 and 4, but without effect. Altogether it had received 3 times the amount of the Morris extract required to kill.

Several factors may have contributed to the failure to obtain positive results:

1. The plants may have belonged to a different species of Eupatorium than E. urticaefolium.

2. They may have failed to develop a poisonous constituent under Minnesota conditions.

3. The Minnesota plants were just coming into bloom, whereas those from Morris, Ill., were just about through that stage—a point to be taken into consideration if we assume for the present that some relation exists, within very narrow limits, between the appearance of the poison and the time of blossoming.

4. The leaf powder may have deteriorated although kept under identically the same conditions as that from Morris.

5. The rabbit may have been naturally immune.

The Solvent Complex

Exper. 27.—While there was little ground, either chemical or physiologic, for believing the fatal results obtained in the foregoing experiments were due to a poisonous compound formed from the solvents themselves, we have, nevertheless, checked this point still further by extracting 45 gm. of Esclepias verticillata (whorled milkweed) with the ether-chloroform-ammonia mixture just as was done with the leaf powder. The residue was fed in 3 doses at 24-hour intervals to Rabbit 21 with no deleterious effect whatever.

This result, together with those of similar experiments in which the same solvents were employed as in Expers. 20, 21, 22, 23 and 24, make it obvious that the solvent complex plays no part in the action of the extracts.

Feeding Experiment with Rabbit Tissues

Exper. 28.—As mentioned elsewhere, the literature on milksickness makes frequent reference to the danger from using the meat and milk of animals affected with the disease. Experimental evidence has been produced which seems to indicate that such statements are not wholly without foundation.

An opportunity of carrying out work along this line was afforded us with the carcasses of rabbits that died in the laboratory from Eupatorium poisoning. On Feb. 16, 1918, a half-grown cat, weighing 2,505 gm. was fed the heart, lungs, liver, kidneys and spleen of Rabbit 14 which died from the alcoholic extract of leaf powder. On the two following days it ate 470 gm. of the meat. On the 20th, it was given the heart, lungs, liver, kidneys and spleen of Rabbit 13, which died from the ether-chloroform-ammonia extract. On the three following days it ate 672 gm. of meat from this animal. No injurious effects were noted. On March 4 it received the heart, lungs, liver, kidneys and spleen of Rabbit 16 (E. C. A. extract) and on the 2 following days 318 gm. of the meat. On March 8, it was

given the viscera enumerated above and 95 gm. of meat from Rabbit 18 (E. C. A. extract); on the next day, 175 gm. meat from Rabbit 18 and the viscera of Rabbit 15 (alc. extr.); on the two following days it ate 438 gm. of meat from Rabbit 15. On March 19, it was given the viscera of Rabbit 11 (E. C. A. extr.) and on the 4 following days a total of 680 gm. of the meat. On April 9, it ate the viscera together with 100 gm. of meat from Rabbit 20 (E. C. A. extract); during the next 2 days, it received 362 gm. of the meat. Once more, on April 15, it was given the heart, lungs, liver, kidneys and spleen and 150 gm. of the meat from Rabbit 8 (Greenhouse Eupatorium); on the next 2 days it consumed the remainder of the meat which amounted to 387 gm.

While being fed on the rabbit tissues, the cat received nothing else but pasteurized, sweet milk, and between times, it was given fresh raw beef and sweet milk. Altogether, the animal consumed the viscera indicated and the muscle tissue of 8 rabbits—the meat alone amounting to 3,970 gm. or more than one and one-half times its own body weight.

The cat was playful all of the time and remained perfectly healthy and normal thruout the experiment. It increased in weight from 2,505 gm. on Feb. 2, to 3,066 gm. on April 17—a net gain of 561 gm. in 59 days.

FEEDING EXPERIMENTS WITH EXHAUSTED RESIDUES

In order to determine whether the leaf powder had yielded its poisonous principle to the different solvents employed, and as a further check on the toxicity of the resulting extract, the leaf residues from the different extractions were fed to rabbits.

RESIDUE FROM 95% ALCOHOL EXTRACTION

Exper. 29.—Rabbit 12 was fed the dried residue from 30 gm. of extracted leaf powder, mixed with chopped carrots, at the rate of 2 gm. per day for 15 days. The animal ate the residue readily and with apparent indifference to its presence in the food.

There was no indication of any injurious effect at any time, altho the total quantity consumed represented more than two lethal doses before extraction for an average rabbit. The extract from this material was fed to Rabbit 14 (Exper. 10) and resulted fatally.

It appears from this that the 95% alcohol had removed the active principle and left the powder impotent.

RESIDUE FROM ETHER-CHLOROFORM-AMMONIA EXTRACTION

Exper. 30.—Twenty-one days after the close of the preceding experiment, Rabbit 12 was given the dried residue from 50 gm. of extracted leaf powder, mixed with chopped vegetables, at the rate of 2 gm. per day. It ate the material readily and retained a good appetite and its normal condition without any manifestation of ill effects whatever.

In this case, the animal ate the residue from more than 3 lethal doses of the extract without harmful results, whereas the extracts when fed to Rabbits 13, 16 and 18 proved fatal (Expers. 15, 16 and 17).

Clearly, the extraction with ether-chloroform and ammonia had removed the active ingredient and left the residue inert.

RESIDUE FROM SALT SOLUTION EXTRACTION

Exper. 31.—An attempt was made in this instance to feed Rabbit 13 with the residue from the salt solution extraction, but with practical failure. The animal refused to eat anything which carried the residue, and after 8 days of persistent trial, during which period the rabbit lost 400 gm., the experiment was discontinued.

The fact that the extract from this material could not be proven to be poisonous (Exper. 14) and that the rabbit showed such a decided dislike for the powder, suggests that the latter still contained an active ingredient which had not been removed by the salt solution.

ORIGINAL UNTREATED LEAF POWDER

Exper. 32.—At the same time that we were conducting Expers. 29 and 31, we endeavored to feed Rabbit 11 with the original potent leaf powder which had been employed earlier in Expers. 4, 5, 6, 7 and 8 with fatal results. The animal refused all food which contained the material and ate almost nothing for 8 days; accordingly the experiment was discontinued.

One cannot help being impressed with the fact that the rabbits seem to be able to distinguish between the residues from which the poisonous ingredient has been removed and those from which no demonstrable poison has

been secured. Whether they are able to discern this difference by intuition, taste or smell is difficult to say, but the fact remains unchanged, that they do eat the residues from which a demonstrable poisonous ingredient has been extracted and refuse all others.

FEEDING EXPERIMENTS WITH GUINEA-PIGS AND EUPATORIUM LEAF POWDER

Exper. 33.—It was shown in a number of our earlier experiments that the leaf powder from Morris, Ill., is poisonous for rabbits; its action upon guinea-pigs was studied by feeding Pig 3, 50 gm. in 2-gm. doses over a period of 25 days. It ate the powder readily when mixed with chopped carrots—quite the opposite from rabbits. Its weight decreased but little more than the control during the experimental period, and no apparent injury followed the ingestion of the snake-root. Weight at the beginning, 475 gm.; at the end, 391 gm.; loss, 84 gm.

Exper. 34.—This is a duplicate of the last experiment. In this case Pig 4 was given 50 gm. leaf powder in 2-gm. portions at 24-hour intervals. It remained perfectly well thruout the feeding, and at the conclusion of the experiment, no harmful effect of the powder could be noted.

Weight at the beginning, 489 gm.; at the end, 421 gm.; loss, 77 gm.

The last two experiments are of particular interest in that they confirm the results of Exper. 26, wherein a guinea-pig was fed the ether-chloroform-ammonia extract from 45 gm. of leaf powder (sufficient to kill 3 rabbits) without any apparent injury.

The fact seems well established from these results that the dried Eupatorium leaves contain nothing which is poisonous for guinea-pigs.

Exper. 35.—Pig 2 was carried as a control in connection with the two preceding experiments. It received the same kind and amount of food as Pigs 3 and 4 except without the addition of the leaf powder. All 3 animals were on a rather short ration as no more food was offered them than they would eat up completely, for we desired to have all of the leaf powder consumed. Under the conditions of the experiment, this control pig lost 62 gm.

SUMMARY

Both the fresh, green Eupatorium urticaefolium and the dried leaf powder contain an active ingredient which is poisonous for rabbits.

The active poisonous principle is present in plants grown in the greenhouse, as well as under natural out-of-door conditions.

The active constituent is soluble in 95% alcohol, and its solution yields a solid extract on evaporation which is poisonous for rabbits, but not for guinea-pigs.

The active ingredient is soluble in a mixture of ether-chloroform and ammonia, and its solution yields a solid extract on evaporation which is poisonous for rabbits, but not for guinea-pigs.

The active ingredient is not yielded by extraction with physiologic salt solution.

The active principle is present in the leaves, but not, or only sparingly so, in the stems and roots of dried plants.

There is no indication of anaphylaxis.

So far as is shown by these experiments, neither the leaf powder nor the different extracts are poisonous for guinea-pigs.

The viscera and meat from rabbits which had died from Eupatorium poisoning, when fed to a cat, were without harmful action.

No difference in poisonous properties could be noted between plants from a "milksick" and "nonmilksick" area.

The fatal dose of the leaf powder differs with the different animals, ranging from 4-17 gm.; whereas, the ether-chloroform-ammonia extract from 15 gm. given in 3 doses 24 hours apart invariably caused death in 4-6 days.

Rabbits suffering with Eupatorium poisoning usually manifest the first symptoms on the 3rd or 4th day (E. C. A. extr.). They refuse to eat, sit humped up, eyes half-closed and often keep the body swaying from side to side; the respiration is usually shallow, rapid and jerky; on the following day (4th or 5th) a flaccid paralysis of the head, neck and front legs ordinarily develops; this is followed by complete prostration and death in 24-36 hours.

The principal pathologic changes occur in the kidney, liver and heart where fatty degeneration and hyperemia are very marked.

Poured agar plates made from the heart blood, liver and kidneys were invariably sterile.

It is not intended to claim that all cases of disease with the symptoms of trembles or milksickness are due to the ingestion of the toxic substance present in Eupatorium leaves. Jordan and Harris have shown that a disease with similar if not identical symptoms occurs in a region in New Mexico where Eupatorium is not present.